

Multivariate and Phylogenetic Analyses of Larval and Adult Characters of the Editha Complex of the Genus *Lycaena* (Lepidoptera:Lycaenidae)

Gordon F. Pratt¹, David M. Wright², and Gregory R. Ballmer³

Abstract. The Editha Complex of the genus *Lycaena*, *L. editha*, *L. xanthoides*, and *L. rubidus*, are united by biological and morphological similarities which are not shared by other North American *Lycaena*. The specific status of parapatric *L. editha* and *L. xanthoides* has been questioned, since populations with intermediate wing characteristics have been noted. Statistical analyses indicate significant larval character differences among populations of each species. Independent discriminant analyses of adult, first instar, and mature larval character sets of the three taxa, *L. editha*, *L. x. xanthoides*, and *L. x. dione*, gave congruent taxonomic classification with most populations. All but one misclassification involved *L. xanthoides*, and these are believed due to phylogenetic relationships of populations rather than 'gene flow.'

Phylogenetic analyses of 17 populations of the Editha Complex, using 30 morphological characters and *L. heteronea* as an outgroup, indicate that *Lycaena rubidus ferrisi* is probably the most primitive taxon. Both *L. editha* and *L. x. xanthoides* appear to have evolved independently from an *L. x. dione* ancestor, probably through vicariant events caused by the glacial periods of the Pleistocene.

Introduction

Analyses using C¹⁴ dating of Pollen from core samples and macrofossils of pack rat (*Neotoma*) middens from various regions of western North America, have revealed great changes in plant biogeography during the Pleistocene (Martin & Mehringer, 1965; Wells, 1983). For instance, during the most recent Wisconsin glacial, subalpine conifers extended down to basal elevations of the Great Basin; desert vegetation, which was replaced by more mesic plants, moved into lowland pockets and to southern desert refugia. Because of the complex topography of the West, dispersal routes as adaptations to the climatic changes of the Pleistocene have been proposed for a number of western birds, mammals, plants (Wells, 1983) and butterflies (Porter & Shapiro, 1991; Austin & Murphy, 1987). These routes were based on island biogeography, the phyletic relationships of neighboring populations, and the potential routes available from these regions.

The Pleistocene probably greatly affected the evolution and biogeography of the *Lycaena editha* Complex, which consists of *L. editha* (Mead), *L. rubidus* (Behr), and *L. xanthoides* (Boisduval), (Klots, 1936; Ballmer

¹ Entomology and Applied Ecology Department, University of Delaware, Newark, DE 19716

² 100 Medical Campus Drive, Lansdale, PA 19446

³ Department of Entomology, University of California, Riverside, CA 92521

& Pratt, 1989b), and primarily confined to western North America. *Lycaena xanthoides dione* (Scudder), the only member of this group found east of the Rocky Mountains, extends into the Great Plains. Although the three species are regionally sympatric, they are confined to different elevations. For example, along the eastern slopes of the Sierra Nevada, in the vicinity of Sherwin Summit, *L. xanthoides* occurs mostly below 2,000 meters, *L. editha* generally above 3,000 meters, while *L. rubidus* occurs between them from 2,000 to 3,000 meters elevation (Ballmer & Pratt, 1989b; Ballmer & Pratt, pers. obs.). Certainly these elevation limits, like those of the desert and subalpine vegetation, must have changed drastically during the most recent Wisconsin glaciation.

A number of problems complicate phylogenetic studies of the Editha Complex: 1) there is a paucity of good phylogenetic characters, 2) there may be 'gene flow' between taxa creating either intermediate or 'primitive' taxa, and 3) because most of the characters which separate these taxa are continuous rather than discrete, there is a high probability of convergence. Intermediate populations between *L. editha* and *L. xanthoides* have been reported from Mather and Dunsmuir, California (Scott, 1980). Four suspected hybrid specimens with *L. rubidus* have been reported, one with *L. x. dione* and three with *L. editha* (Scott, 1980; Anonymous, 1986; & Crowe, 1970).

Scott (1980) compared different populations of *L. editha* and *L. xanthoides* using 15 wing characters. He found that only two of those 15 characters separated *L. editha* from *L. xanthoides*: wing length and ventral hindwing spot size. The populations from Mather and Dunsmuir, California were intermediate in these two characters. Scott (1980) also determined that *L. x. dione* was the most distinct of the different comparisons, since it differed from both *L. editha* and *L. xanthoides* on the basis of eight wing characters. Unfortunately no statistics were used to determine the significance of these characters.

The use of first instar setal patterns has been proposed for elucidating the phylogenetic relationships of the Lycaenidae (Clark & Dickson, 1956). These characters differ greatly from those of later instars, since first instars have only primary and subprimary setae, whereas in third and fourth instars only secondary setae can be discriminated (Wright, 1983; Ballmer & Pratt, 1989b). Primary and subprimary setae are distinguished from secondary setae by their numbers and positions, which are fixed in specific locations on the larva; secondary setae are variable in both number and location (Ballmer & Pratt, 1989b). Many of the morphological structures found in mature *Lycaena* larvae are not found in first instars, such as mushroom, dendritic, and other specialized setae (Ballmer & Pratt, 1989b). Mature larval characters, representing the opposite end from that of first instars of a spectrum of characters expressed in the larval stage, are useful in distinguishing *L. editha* from *L. xanthoides* (Ballmer & Pratt, 1989b).

The two closest relatives to the Editha Complex are *Lycaena heteronea* (Boisduval) and *L. gorgon* (Boisduval). In fact these two species are so

morphologically similar to the Editha Complex that Miller and Brown (1979) misrepresented their phylogenetic relationships. They placed *L. rubidus* with *L. heteronea* in the genus *Chalceria*, and *L. editha* and *L. xanthoides* along with *L. gorgon* in *Gaeides*, even though male genitalic structures indicate that *L. gorgon* and *L. heteronea* belong to one group, while members of the Editha Complex comprise another species group (Klots, 1936). Details of larval biology and morphology support this genitalic arrangement of the species (Ballmer & Pratt, 1989b).

In this paper we employ discriminant and phylogenetic analyses of larval and adult wing characters to investigate the status of the different species of the Editha Complex, with emphasis on *L. editha* and *L. xanthoides*. *Lycaena heteronea* was used as an outgroup comparison because it shares the presence of dendritic setae on the mature larva with that of the Editha Complex; these setae are lacking in all other North American Coppers (Pratt, Ballmer, & Wright, pers. obs.). Based on various phylogenies of the taxa at a population level, we hypothesize the different dispersal routes and vicariant events which may have led to the historical evolution of the different members of this complex.

Materials and Methods

OVIPOSITION AND REARING

Larvae were primarily reared from ova obtained from captive females; some were field collected. Females of *L. x. xanthoides* from Mojave River Forks were reared from larvae and mated in the lab (as in Ballmer and Pratt, 1989a); mated females of other populations were field-collected. Dried leaves of *Rumex acetosella* L., *Rumex crispus* L., and *Rumex salicifolius* Weinm. were used as oviposition substrates. Egg diapause was terminated by refrigeration at 4°C for 3-5 months (as in Pratt and Ballmer, 1986). Within 7-10 days after removal from 4°C and incubation at 25°C, first instars eclosed and were reared on *R. crispus* until mature. Mature larvae were preserved and injected, as in Ballmer and Pratt (1989b). First instar larvae were primarily obtained by dissection from ova, while those that eclosed were fed *R. crispus* and distended and fixed in first instar distention fluid (10 ml glacial acetic acid, 1 ml glycerin, 0.1 ml Triton X-100®, and 89 ml water).

FIRST INSTAR CHARACTERS

First instar characters (see Fig. 12 in Wright, 1983) are as follows: number of SD1 and SD2 setae on prothoracic shield, of SD2 setae on T2-A7 and A9, of SD3 setae on T2, T3, and A3-6, of SD4 setae on A1-A7, of SD1 setae on A9, of lateral setae and dorsal lenticles on A8; lengths of D1 and D2 setae on T2 and A1, of L1 and L4 setae on T2, L4 seta on T3, and L3 seta on A1; and mean crochet number per proleg on A3-A6. SD3 and SD4 are derived subprimary setae common to lycaenids. Frequently short and clubbed, SD3 is located near proprioceptor MD1 on thoracic and abdominal segments; SD4 is located near the spiracle on abdominal segments. Permanent slide mounts of first instar cuticle were prepared following brief KOH (10%) digestion of internal soft parts and cleansing of cuticle. All characters were analyzed with a binocular microscope (200-400X), and measurements were made with an ocular micrometer. The locations and sample sizes are shown in Table 1 and Figure 1.

Table 1. Locations and Sample Sizes of the Different Populations for First Instar Characters

Taxon	Location*	County	State	N
<i>L. x. xanthoides</i>	Sacramento	Sacramento	CA	9
	Branscomb Lake	Mendocino	CA	6
	Mojave River Forks	San Bernardino	CA	7
	Acton	Los Angeles	CA	9
	Sherwin Summit	Mono	CA	8
	Silver Canyon	Inyo	CA	10
<i>L. x. dione</i>	Idledale	Jefferson	CO	3
	Milford	Seward	NB	12
	Brookfield	Waukesha	WI	6
<i>L. editha</i>	Sagehen Mdws	Mono	CA	8
	Mt Barcroft	Mono	CA	7
	Tioga Pass	Mono	CA	9
	Winter Park	Grand	CO	6
	Dunsmuir	Siskiyou	CA	12
<i>L. r. rubidus</i>	Tioga Pass	Mono	CA	8
<i>L. r. ferrisi</i>	Ditch Camp	Apache	AZ	6
<i>L. heteronea</i>	Chuchupate Cmpgd	Ventura	CA	8
	Warner Valley	Lassen	CA	6

*The locations are shown in Figure 1.

MATURE LARVAL CHARACTERS

Mature larval characters (see Fig. 1 in Ballmer and Pratt, 1989b) are as follows: number of dendritic setae on T1 and A1-A8, number of non-sensory/non-mushroom and mushroom setae on the prothoracic shield, head width, head and leg pigmentation using *L. xanthoides* (Mojave River Forks) and *L. editha* (Tioga Pass) as standards, length of the longest dorsal seta on A1, and mean lateral crochet number on prolegs A3-A6. Measurements were made with an ocular micrometer using a binocular dissecting microscope. The locations and sample sizes are shown in Table 2 and Figure 1.

ADULT CHARACTERS

Adult characters (Figure 2) are as follows: length of the Cu2 vein in the forewing and hindwing; medial width of the dorsal Cu1 aurora; lengths of the dorsal M3 discal and Cu2 basal shadows of the forewing; longest length and perpendicular width of the basal macule on the hindwing; longest lengths of the M1 and M2 discal macules of the hindwing, and of the M3 and Cu1 discal macules of the forewing. These measurements were made with an ocular micrometer using a binocular dissecting scope. The locations and sample sizes are shown in Figure 1 and Table 3.

DISCRIMINANT ANALYSIS

Discriminant Analyses (Hand, 1981) were performed on *L. x. xanthoides*, *L. x. dione*, and *L. editha* using the SAS program. Three reference populations of the

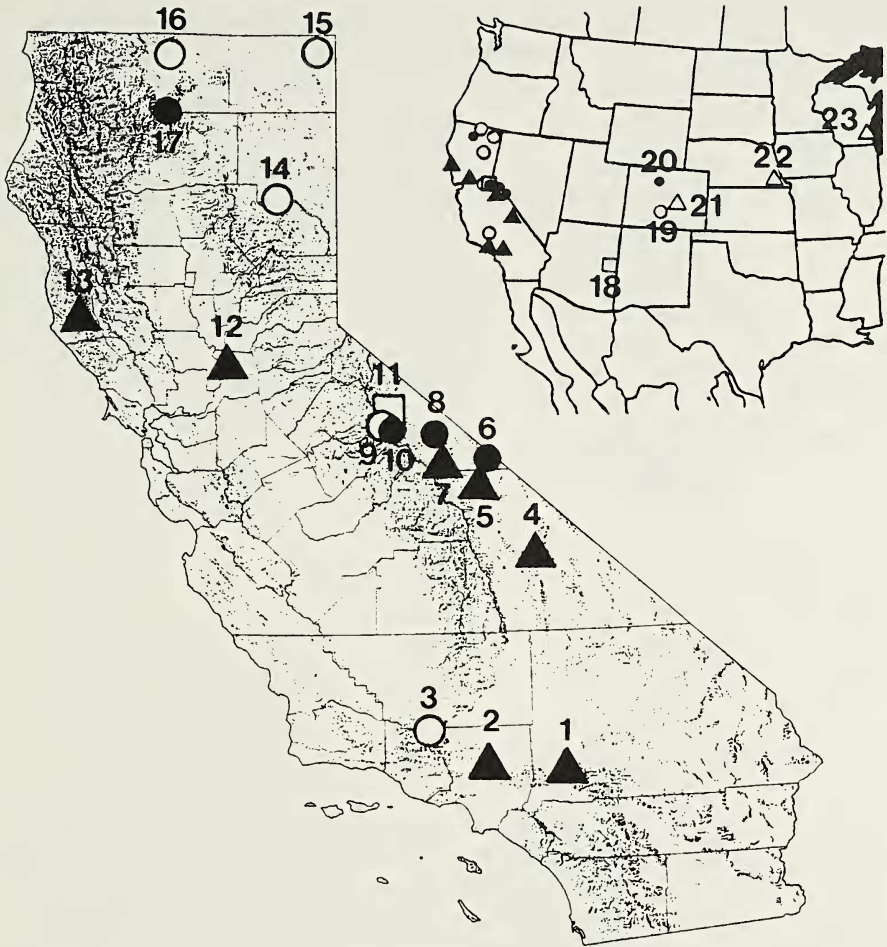


Figure 1 Locations of the various populations of *Lycaena*: open circles = *L. heteronea*, closed circles = *L. editha*, closed triangles = *L. x. xanthoides*, open triangles = *L. x. dione*, and open squares = *L. rubidus*. The locations are as follows: 1=Mojave River Forks, 2=Acton, 3=Frazier Park, 4=Hunter Mt, 5=Silver Canyon, 6=Mt Barcroft, 7=Sherwin Summit, 8=Sagehen Meadows, 9=Tioga Pass, 10=Tioga Pass, 11=Warren Canyon, 12=Sacramento, 13=Branscomb Lake, 14=Mt Lassen, 15=Mt Bidwell, 16=Ball Mt, 17=Dunsmuir, 18=Ditch Camp, 19=Westcliffe, 20=Winter Park, 21=Idledale, 22=Nebraska, and 23=Wisconsin.

taxa were used in these analyses, the remainder were classified (as test unknowns) as to their likely membership into one of these reference populations. Some reference populations had to be lumped with neighboring populations, since their sample sizes were considerably smaller than the number of characters. These reference populations are as follows: *L. x. xanthoides* from Sacramento CA (mature larvae and adult female) and Sacramento and Branscomb Lake (first instar); *L. x. dione* from Nebraska (mature larva), all of the adult females (adult female), and Nebraska and Wisconsin (first instar); and *L. editha* from Tioga Pass and surrounding areas (mature larva and adult female) and

Table 2. Locations and Sample Sizes of the Different Populations for Mature Larval Characters

Taxon	Location*	County	State	N
<i>L. x.</i>				
<i>xanthoides</i>	Sacramento	Sacramento	CA	20
	Mojave River Forks	San Bernardino	CA	15
	Hunter Mountain	Inyo Co	CA	3
	Branscomb Lake	Mendocino	CA	2
	Silver Canyon	Inyo	CA	6
	Sherwin Summit	Mono	CA	4
<i>L. x. dione</i>	Milford	Seward	NB	20
<i>L. editha</i>	Tioga Pass	Mono	CA	16
	Mount Barcroft	Mono	CA	14
	Dunsmuir	Siskiyou	CA	11
<i>L. r. rubidus</i>	Tioga Pass	Mono	CA	9
<i>L. heteronea</i>	Mount Bidwell	Modoc	CA	11
	Warren Canyon	Mono	CA	4
	Frazier Mountain	Kern	CA	2
	Westcliffe	Custer	CO	15

* The locations are shown in Figure 1.

Tioga Pass and Mt. Barcroft (first instar). Four characters were removed from the first instar analysis: the number of the SD1 and SD2 setae on the prothoracic shield and the number of SD3 and SD2 setae on the metathorax and the ninth abdominal segments, respectively. These setae are commonly absent on both *L. editha* and *L. xanthoides*; their presence or absence is more important in discriminating these taxa from *L. rubidus* and *L. heteronea*.

PHYLOGENETIC ANALYSIS

All of the morphological characters of the different populations were statistically compared using the general linear models procedure of SAS. Different statistical classes were defined using a T-test ($P < 0.05$) for each morphological character for the different populations. Populations within each class were significantly different from every other population outside of the class. The number of classes were maximized and ordered according to decreasing magnitude of the means of the morphological character. The first class was given a value of one, the next class a value of 2, etc. Two additional adult characters (iridescence and color of the male dorsal wing surface) were added to the phylogenetic analysis. These two characters were discrete and coded without statistics. The iridescence was coded as present=2 or absent=1. The color gray was coded as 1, orange as 2, and blue as 3.

These morphological scores were analyzed by PAUP (Phylogenetic Analysis Using Parsimony, version 2.4, Swofford, Illinois Natural History Survey). Mulpars and global branch swapping were performed with the FARRIS optimization. Since the characters were continuous, the Weights Scale option was used.

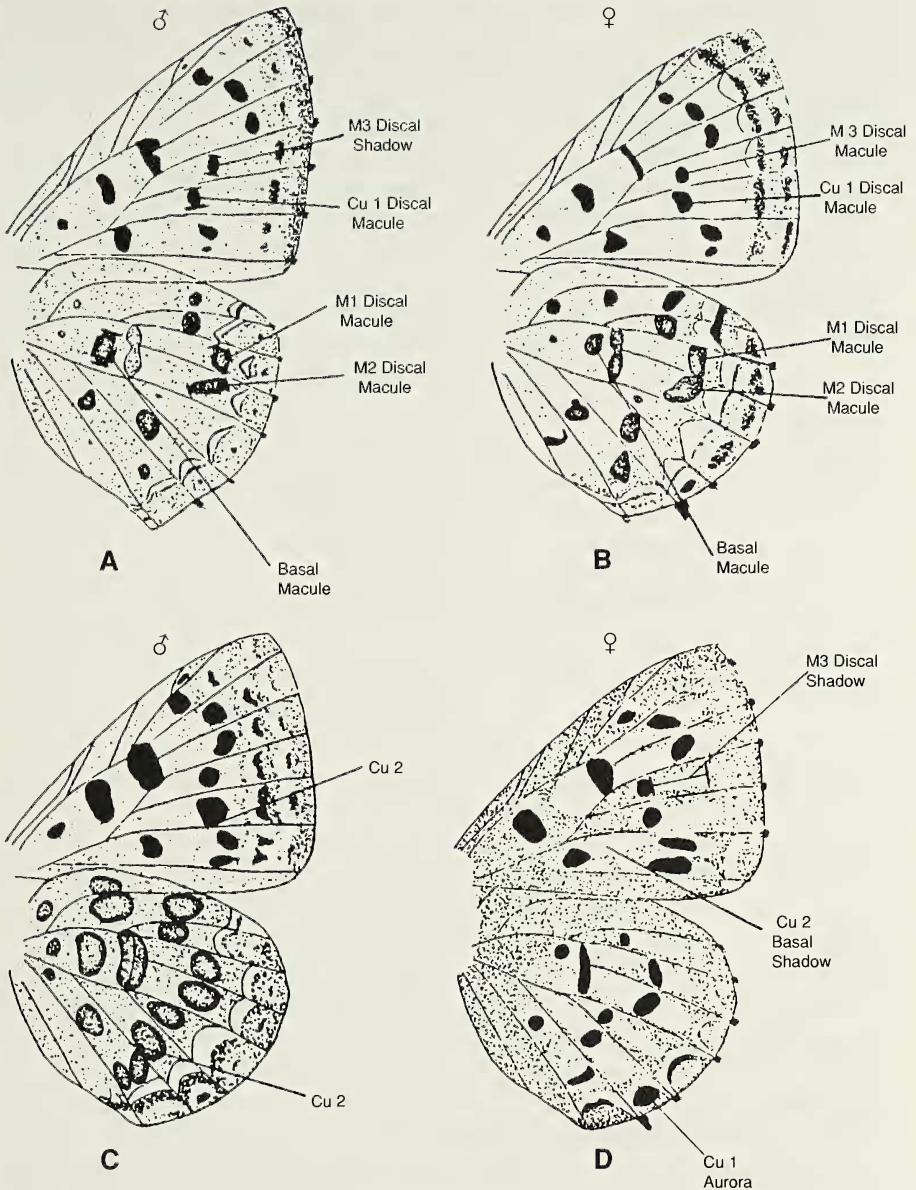


Figure 2 A) Ventral wings of male *Lycaena editha* from Dunsmuir. Cu1 is cubital vein 1, M1 is the medial vein 1, M2 is the medial vein 2, M3 is the medial vein 3. B) Ventral wings of female *L. editha* from Dunsmuir. C) Ventral wings of female *L. editha* from Mt Barcroft, White Mts. Cu2 is the cubital vein 2. D) Dorsal wings of female *L. editha* from Dunsmuir.

Lycaena heteronea was used as an outgroup in most analyses, so in these cases no prior assumptions were made about the polarity of character states (primitive versus advanced). Those character states which were closest to that of *L. heteronea* were therefore accorded primitive status by the analyses.

Table 3. Locations and Sample Sizes of the Different Populations for Adult Female Characters

Taxon	Location*	County	State	N
<i>L. x. dione</i>	Winnipeg	Manitoba	2	
	Omaha	NB	6	
	Blue Springs	Jackson	MO	2
	Lees Summit	Jackson	MO	2
<i>L. x. xanthoides</i>				
Sacramento area	Stone Lakes	Sacramento	CA	9
	West Sacramento	Yolo	CA	1
	American R. Parkway	Sacramento	CA	10
<i>L. x. xanthoides</i>				
Southern CA area	Lake Henshaw	San Diego	CA	1
	Mojave R. Forks	San Bernardino	CA	3
	Solemint	Los Angeles	CA	1
	Vista Grande			
	Ranger Station	Riverside	CA	1
	Tahquitz Lodge	Riverside	CA	1
<i>L. x. xanthoides</i>				
	Los Altos	Santa Clara	CA	4
<i>L. x. xanthoides</i>				
	Sherwin Summit	Mono	CA	2
<i>L. x. xanthoides</i>				
	Hunter Mt	Inyo	CA	8
<i>L. editha</i>				
<i>editha</i>	Tioga Pass	Mono	CA	17
	Dunsmuir	Shasta	CA	12

* The locations are shown in Figure 1.

Results

THE MORPHOLOGICAL CHARACTERS

The Editha Complex separates from *Lycaena heteronea* on the basis of two first instar characters, the crochets and D2 length on A1. None of the taxa of the Editha Complex separate as clearly from the remainder. *Lycaena rubidus* is significantly different from *L. editha*, *L. x. xanthoides*, and *L. x. dione* (but not from *L. heteronea*) on the basis of the prothoracic shield SD1 and SD2 setae. *Lycaena. x. xanthoides* separates from the other taxa on the basis of the D1 and D2 setal lengths on A1. Because of variability, both *L. editha* and *L. x. dione* are only significantly different for one first instar character (L3 on A1). This character separates *L. x. dione* from *L. x. xanthoides* and *L. editha*, but not from *L. rubidus* and *L. heteronea*.

Three mature larval characters separate *L. heteronea* from members of the Editha Complex: the number of dendritic setae on A1, the ratio of the number of dendritic setae on A7 to A8 and the length of the dorsal setae on A1. Within the Editha Complex, *L. r. rubidus* (Warren Canyon) is distinct from all other populations in the number of dendritic setae on A3-6 and non-sensory/non-mushroom (secondary) setae on the prothoracic shield. *Lycaena editha* populations are distinct from most *L. x. xanthoides* in having more dendritic setae on A2 and a darker head capsule and legs.

In addition to smaller wing size, two adult characters separate *L. editha* from *L. xanthoides*: the ratio of the length of the hindwing M2 discal macule to the forewing Cu1 discal macule (>1 in *L. editha* but <1 in *L. xanthoides*) and the width of the dorsal orange aurora along Cu1. Of these two characters only the macule ratios yield significantly distinct classes as discussed in the Materials and Methods. The mean ratio of the M2 macule to the Cu1 macule is less than or approximately equal to one in *Lycaena gorgon*, *L. nivalis* (Boisduval), *L. heteronea*, *L. rubidus*, *L. phlaeas* (L.), and *L. xanthoides* (Pratt, pers. obs.). The mean ratios for *L. editha* specimens from Tioga Pass and Dunsmuir were 1.33 and 1.54, respectively, and were significantly different from the other *Lycaena*, and each other. In *L. x. dione* the orange aurora is wider than in three of the five *L. x. xanthoides* populations, but is similar to that of the Hunter Mt and Southern California populations.

In discriminant analyses all individuals of the reference populations were classified in posterior tests to their proper taxon, showing that the discriminant functions properly discriminate these three taxa. Mature larval characters gave the best discrimination of the test unknowns. Out of 55 unknowns, only two misclassifications were obtained, two *L. x. xanthoides* larvae from Silver Canyon were misclassified as *L. x. dione*. The first instar analysis misclassified 11 out of 63 unknowns. Two of three *L. x. dione*, and two of eight and seven of ten *L. x. xanthoides* from Sherwin Summit and Silver Canyon, respectively, were misclassified as *L. editha*. Out of 33 adult female unknowns, nine were misclassified, one of 12 *L. editha* from Dunsmuir was misclassified as *L. x. xanthoides* and eight *L. x. xanthoides* were classified as *L. x. dione* (5 of 8 from Hunter Mt and 3 of 7 from Southern California). Complete data of the morphological characters can be obtained from the first author.

PHYLOGENETIC ANALYSIS

Twenty eight morphological characters formed significantly different classes and were used in the phylogenetic analyses along with two discrete adult wing characters. Some continuous characters did not form significantly different classes as defined in the Materials and Methods, although there were significant differences amongst the populations.

The phylogenetic trees produced by PAUP are based on the Wagner Algorithm, which assumes that the best estimate of a phylogenetic tree is that which has the fewest character changes (most parsimonious).

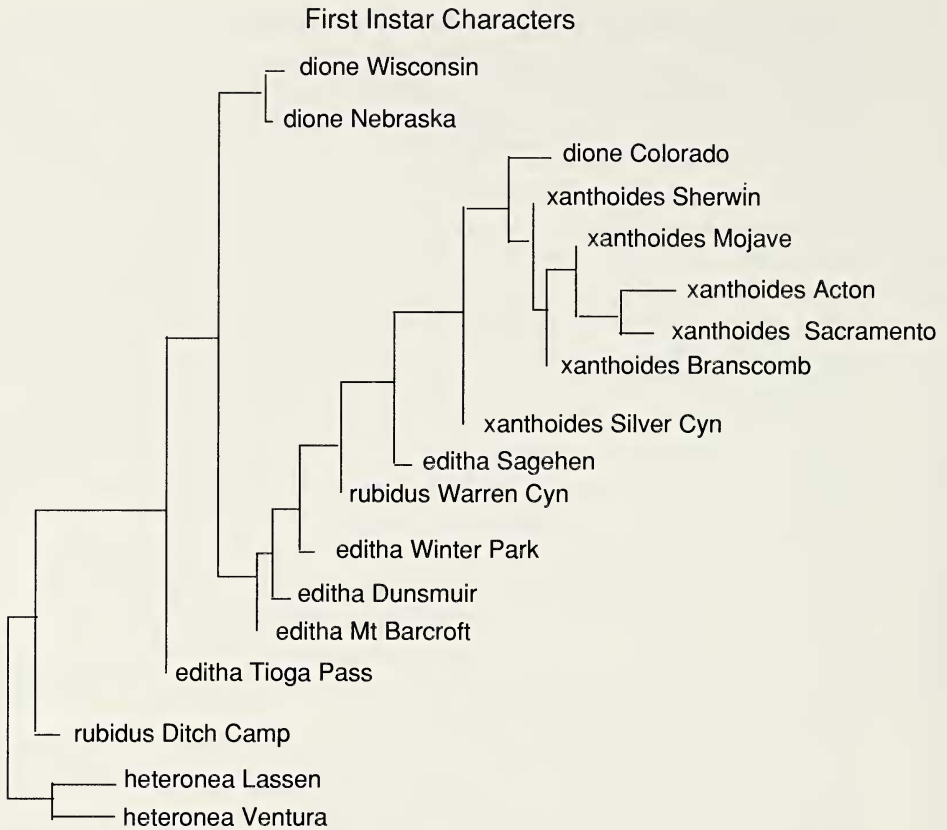


Figure 3 Phylogenetic Analysis (Distance Wagner Tree) of the members of the *L. editha* complex using first instar characters and *L. heteronea* as an outgroup. Length is 22.05 and the consistency index is 0.590.

Wagner trees show the interrelationships amongst the operational taxonomic units (populations) better than UPGMA derived trees, since they do not assume that taxonomic units exhibit equal evolution through time. In other words some populations can be more primitive (plesiomorphic) than others. Not only are the interbranch lengths important with these phylogenies, but so too are the branch lengths of the individual taxonomic units, since they indicate how advanced (apomorphic) the units are. From an analysis of nine taxonomic units, slightly more than 4,000 different phylogenies or arrangements of the units are possible. This number increases geometrically with the number of taxonomic units being investigated. It is not surprising, therefore, that there may be many phylogenies which are equally parsimonious in an analysis of more than nine taxonomic units.

Three equally parsimonious trees were produced from the 14 first instar characters, their topologies, or branching structures, were identical to Figure 3. All populations of *L. x. xanthoides* and *L. x. dione* (Colorado) were advanced and clustered together, whereas *L. r. ferrisi* (Ditch Camp) and *L. editha* (Tioga Pass) were primitive. *Lycaena r.*

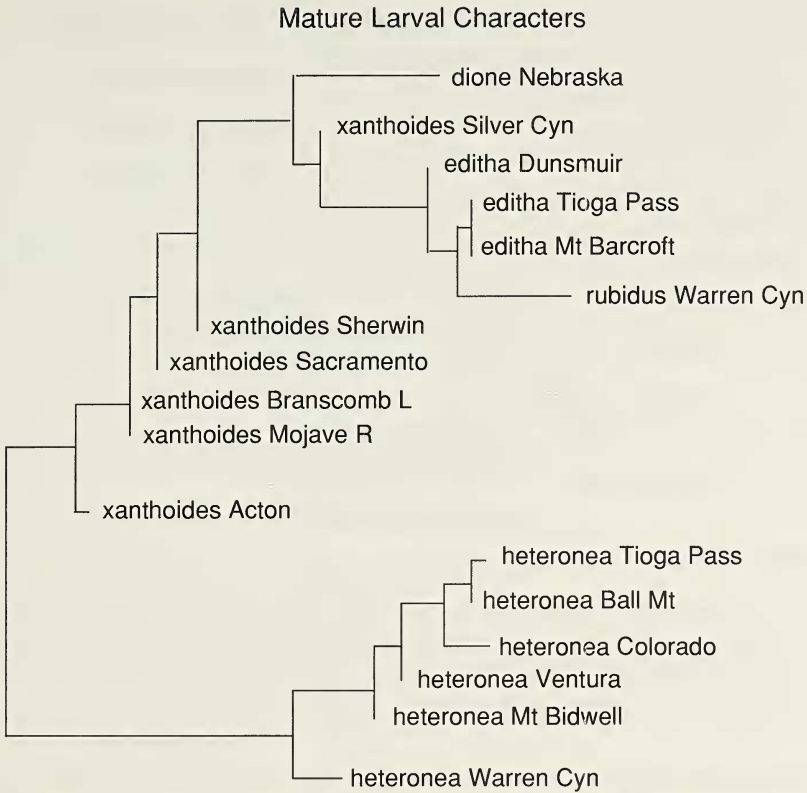


Figure 4 Phylogenetic Analysis (Distance Wagner Tree) of the members of the *L. editha* complex using mature larval characters and *L. heteronea* as an outgroup. Length is 15.65 and the consistency index is 0.767.

rubidus (Warren Canyon) was advanced compared to *L. r. ferrisi* (Ditch Camp) with respect to first instar characters, and clustered amongst the *L. editha* populations. The other populations of *L. x. dione* and *L. editha* were intermediate, yet did not cluster together. The Colorado *L. x. dione* population appears to be distinct from the two other *L. x. dione* populations and is more closely related to *L. x. xanthoides* than is *L. editha*. *Lycaena editha* (Sagehen Meadows) and *L. xanthoides* (Silver Canyon), were the most advanced and most primitive of their species, respectively.

Twenty-one phylogenies were constructed from the 13 mature larval characters. All showed *L. xanthoides* as most primitive, with *L. editha* as intermediate, and *L. rubidus* as the most advanced species (Figure 4). *Lycaena x. dione* clustered closely with *L. x. xanthoides*, but as more primitive. The major differences amongst the 21 trees were the precise arrangement amongst the *L. xanthoides* populations. The arrangement of *L. xanthoides* Silver Canyon was the same with respect to *L. editha* and *L. rubidus*, in all 21 trees.

These phylogenies also show an interesting relationship amongst the *L. heteronea* populations. Those *L. heteronea* populations which exhibit

Mature Larval Characters with Dark Legs and Head as Primitive

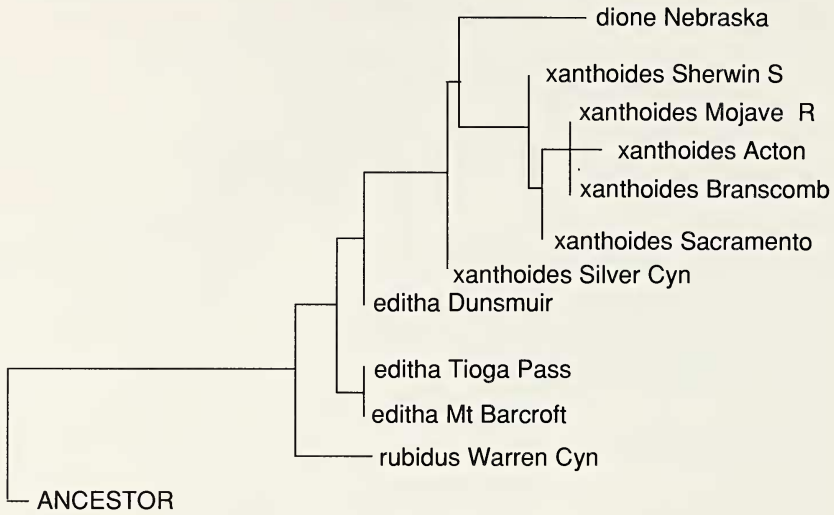


Figure 5 Phylogenetic Analysis (Distance Wagner Tree) of the members of the *L. editha* complex using mature larval characters and a hypothetical ancestor (characters of *L. heteronea*, but with a dark head capsule and legs). Length is 14.4 and the consistency index is 0.764.

well formed macules on the ventral hindwings (Tioga Pass, Ball Mt, and Colorado) cluster separately from those with no visible macules on the ventral hindwings. The Tioga Pass *L. heteronea* (on *Eriogonum nudum* Doug. ex Benth.) was less than 1 km from the Warren Canyon population of *L. heteronea* (on *Eriogonum umbellatum* Torr.). The same two populations also differ in other morphological features and in ant attendance (Ballmer and Pratt, 1992).

Since the phylogenies derived from mature larval characters differed dramatically in the placement of *L. rubidus* from those derived from first instar characters, phylogenies were constructed with a hypothetical ancestor modified from *L. heteronea*. This was to test whether the polarization of the taxonomic units could be easily reversed by changes in character states to those which could have been primitive for the complex. The two partially correlated characters, the dark coloration of the head capsule and legs, are quite variable amongst the Lycaenidae. Populations of the same species can differ in these two characters (Ballmer & Pratt, 1989b). A hypothetical ancestor (with a dark head capsule and legs) causes the polarity of the taxa to be completely reversed (Figure 5).

Since phylogenies can be affected easily by only a couple of changes in the polarity of character states, a single convergence in a character state could cause a reversal in the polarity of a phylogeny. The more characters used in a phylogenetic analysis, the more robust the analysis will be and the less likely that a single character will have this affect. Therefore a

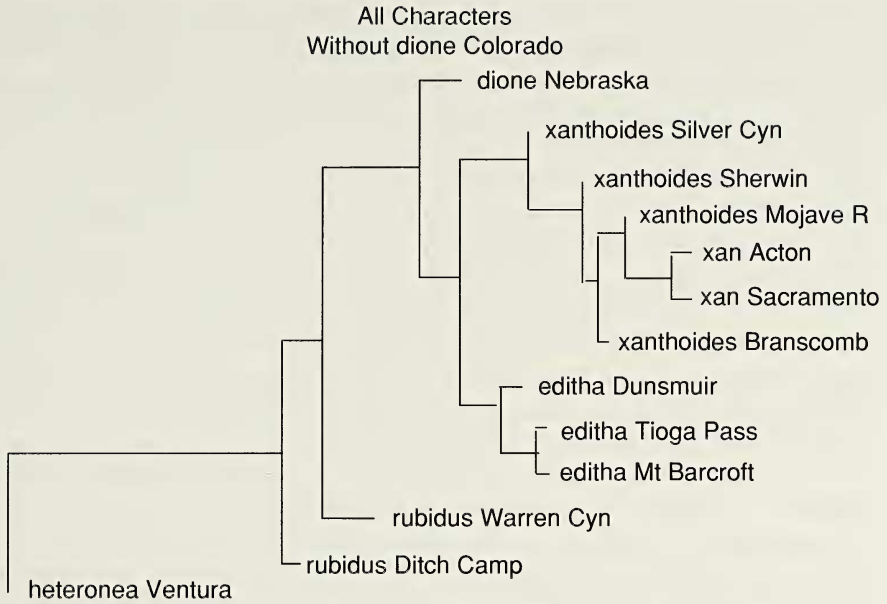


Figure 6 Phylogenetic Analysis (Distance Wagner Tree) of the members of the *L. editha* complex using first instar, mature larval, and adult characters and *L. heteronea* from Ventura as an outgroup. Length is 43.726 and the consistency index is 0.663.

phylogeny was constructed using all 30 morphological characters and *Lycaena heteronea* (Ventura) as an outgroup. Since mature larval characters were not available for *L. r. ferrisi* (Ditch Camp), these characters were coded as missing. The *L. x. dione* Colorado population was not included, since it was based on only three first instar larvae. Only one Wagner Tree was produced from this analysis (Figure 6). *Lycaena rubidus ferrisi* (Ditch Camp) was the most primitive taxon amongst the Editha Complex. All of the *L. x. xanthoides* populations clustered together, as did all *L. editha*, and the two *L. rubidus* populations clustered at the base. This phylogeny suggests that *L. x. dione* gave rise to *L. editha* and *L. x. xanthoides* independently.

DISCUSSION

Since the number of characters used for the first instar and mature larval derived phylogenies were small, 14 and 13 respectively, there are a number of possible explanations of why the two phylogenies were quite different. One of these, convergence of character states with the outgroup was examined (Figure 5) and may be a partial explanation. Another explanation is that different operational units were examined in the two analyses. It would also seem likely that the mature larval characters contained different evolutionary information from that of first instars and that an analysis including all of the characters, would be a more robust representation of the historical evolution of the complex.

Some first instar characters may be correlated with mature larval characters, such as lengths of primary and secondary setae. By combining these character sets an uneven weighting due to these correlated characters would be obtained in a final analysis. However, with *Euphilotes* Mattoni a correlation of setal lengths in first instars to mature larvae appears unlikely, since all combinations in lengths have been observed (Pratt, 1988). *Euphilotes enoptes mojave* (Watson & Comstock) first instars have very short primary setae, while mature larvae have long dorsal secondary setae. *Euphilotes enoptes ancilla* (Barnes & McDunnough) is the reverse with long primary setae and short dorsal secondary setae. *Euphilotes enoptes dammersi* (Comstock & Henne) has both short primary and secondary setae, whereas *E. battoides baueri* (Shields) has both long primary and secondary setae.

Within the Editha Complex there are three closely related entities (*Lycaena rubidus*, *L. xanthoides*, and *L. editha*). Recently, Scott (1980) reduced *L. editha* to a subspecies of *L. xanthoides*. The Dunsmuir *L. editha* population was proposed by Scott (1980) as intermediate between these taxa. In this study we find that although the Dunsmuir population is intermediate in wing size, it is not with larval and adult characters. With discriminant analyses of these characters, the Dunsmuir population clearly classifies as an *L. editha*. Also, a character which separates all adult *L. editha* from other *Lycaena* (ratio of macule lengths) is not intermediate in the Dunsmuir population.

Scott (1980) found only two morphological characters that distinguished *L. editha* from *L. xanthoides*; these characters were the size of the wings and the macules. He showed that the M2 macule size for the Dunsmuir population is intermediate between *L. editha* and *L. xanthoides*. This may be due to the way the macule was measured (parallel to a wing vein), rather than its maximum length. Scott's method is satisfactory when the M2 macule is circular or when it is elongate and its long axis is parallel to vein M2, as in *L. editha* from the White Mountains (Figure 2D). However, Scott's method gives an erroneous value for macule width in specimens with elongate macules whose long axes are not parallel to the vein, as often found in *L. editha* from Dunsmuir (see Figure 2C).

From the phylogeny based on first and last instar larvae and adults (Figure 6), both *L. editha* and *L. x. xanthoides* appear to have evolved from *L. x. dione*. The depicted arrangement of populations within and between taxa, may also reflect an historical pattern of evolution. For instance, in the area where *L. x. xanthoides* shared the most recent connection with extant *L. x. dione* populations, the latter could be more similar to *L. x. xanthoides* than are other *L. x. dione* populations. Therefore, considering the geographic distributions of the two taxa and of the populations in this study (Figure 1), *L. x. dione* from Colorado (the most southwestern) should be closest phenetically to *L. x. xanthoides* from Silver Canyon, Sherwin Summit, and Hunter Mountain (the most eastern). The *L. x. dione* population from Colorado is closest to *L. x.*

xanthoides from Sherwin Summit and Silver Canyon according to a first instar phylogeny (Figure 3), which is the only complete data set for the three *dione* populations. Discriminant analyses of mature larvae from Sherwin Summit and adults from Hunter Mountain misclassify 2 of 6 larvae and 5 of 8 adults, respectively as *L. x. dione*. This supports the hypothesis that *L. x. xanthoides* evolved from or was founded by *L. x. dione*, rather than by *L. editha*.

The elevation differences between *L. editha* and *L. xanthoides* populations in the Sierra Nevada and Inyo Mountains generally make the two taxa asynchronous in flight periods. Along the east slope of the Sierra Nevada *L. xanthoides* occurs from about mid-May to mid- or late June at 2,000 meters, whereas *L. editha* generally doesn't occur before early to mid-July at 3,000 meters. Although it is possible that an early male of *L. editha* may encounter a late female of *L. x. xanthoides*, by that season the *L. x. xanthoides* female should have been mated.

Still 'gene-flow' may be occurring between *L. editha* and *L. xanthoides* at Silver Canyon and perhaps Sherwin Summit, since according to a discriminant analysis of first instars they are phenetically closer to *L. editha* than are other *L. x. xanthoides* populations. It is also possible that these relationships are due to retention of plesiomorphic characters, since according to discriminant analyses two of three first instars of *L. x. dione* from Colorado, also misclassify as *L. editha*. In any case, these populations cluster more closely with *L. xanthoides* than with *L. editha* (Figures 3-6).

Lycaena heteronea was chosen as an outgroup because it belongs to the most closely related group (along with *L. gorgon*) to the Editha Complex, and is the only other North American *Lycaena* known to possess dendritic setae in later instars (Ballmer & Pratt, 1989b). The mature larval characters which make *L. xanthoides* appear close to *L. heteronea* may be due to retained plesiomorphic characters, convergent derived characters, or a combination of both (Figure 4). However, additional characters of mature larvae shared by *L. heteronea* and *L. gorgon* are absent in the Editha Complex and all other North American *Lycaena* (Ballmer & Pratt 1989b). These character states and those of first instar body and cranial chaetotaxy (which are shared with other North American Coppers) indicate that the Editha Complex is more plesiomorphic (older) than *L. heteronea* (Wright pers obs.).

Although there is a possibility that *L. xanthoides* and *L. editha* may be exhibiting some regional or site-specific gene-flow, all populations examined can be assigned to one or the other species in the complex. With respect to larval characters, *L. rubidus* appears to be at least as close to *L. editha* as it is to *L. xanthoides*. There does not appear to be continuous blending of populations of *L. xanthoides* from the south or low elevations with *L. editha* in the north or high elevations. Isolated populations of *L. editha* from Tioga Pass (Sagehen Meadows, Winter Park, and Mt Barcroft) cluster with other *L. editha* populations and isolated populations of *L.*

xanthoides (Hunter Mt, Silver Canyon, and Sherwin Summit) cluster with other *L. xanthoides* populations. It is possible that there are narrow blend zones between the species, but at present these have not been identified.

Lycaena rubidus ferrisi is more primitive and significantly different from *L. r. rubidus* (Warren Canyon) on the basis of seven of 14 first instar characters used in the phylogenetic analysis. Perhaps these two taxa are separate species as has been proposed by Johnson and Balogh (1977). This distinction may also be due to either a sample bias or more likely to the geographic isolation between the two taxa. More populations of *L. rubidus* need to be sampled in order to arrive at a species level decision regarding these two taxa.

DISPERSAL ROUTES AND VICARIANT EVENTS OF THE EDITHA COMPLEX

Lycaena rubidus

Lycaena rubidus ferrisi was shown to be the most primitive taxon in the Editha Complex. This is particularly interesting since this taxon is presently allopatric with all other North American *Lycaena* (Scott, 1986). The formation of *L. r. ferrisi* therefore could have been the initial vicariant event which separated the Editha complex from its sister taxon and allowed it to evolve in isolation.

The dispersal routes open to *L. r. ferrisi* for the subsequent formation of *L. rubidus* at Warren Canyon could have been long and precarious. The species may have moved through the mountain ranges to the north of where *L. r. ferrisi* occurs (WH in Figure 7) into Colorado, Wyoming, Montana, and then westward to the Cascades of Washington and Oregon and south into the Sierra Nevada. This movement could have resulted in a series of founder events which caused the differentiation of *L. rubidus* at Warren Canyon. Since intermediate populations of *L. rubidus* were not used in the first instar phylogenetic analysis, it is not surprising that the character steps of this population would be more parsimonious with other derived populations of other taxa. This phylogenetic discrepancy disappeared with a more robust analysis.

L. x. dione

The most likely method by which *L. x. dione* formed from *L. r. ferrisi* is during a glacial period, perhaps the first of the four which occurred during the Pleistocene. This would have caused *L. r. ferrisi* to move down in elevation, perhaps to the Colorado Plateau (C in Figure 7). When the glacier retreated, the Colorado Plateau, where no *Lycaena* presently resides, probably became more xeric and an unfit habitat for the food plants, *Rumex* sp. Perhaps some individuals retreated up the mountains, whereas others at the eastern edge of the plateau went east into the Great Plains (GP in Figure 7). At least this Colorado Plateau population would have become adapted to Prairie habitats during the glacial period. Since



Figure 7. The map illustrates the present geographic regions of grasslands and mountains of western North America. The dark areas represent mountain ranges and the grasslands are outlined, while the remaining areas of the West are largely desert. The following abbreviations: C=Colorado Plateau; CR=Colorado Rocky Mountains; GB=Great Basin Desert; GP=Great Plains; I=the edge of the Glacier during the Wisconsin Glacial period; IN=Inyo and White Mountains of California; M=Mojave Desert; S=Sierra Nevada; SV=Sacramento Valley and San Joaquin Valley; W=Wasatch Range; WH=White Mountains of Arizona; XR=the present southern limit of *L. x. dione*.

the present southern limit of the range of *L. x. dione* (XR in Figure 7) is north of the Colorado Plateau, *L. x. dione* must have moved north as the glacier retreated, which may have further isolated *L. rubidus* from *L. x. dione*.

L. editha

Two biological adaptations must have occurred in the ancestral *L. x. dione* which formed *L. editha*: first it became adapted to high elevations and/or cool environments; second, and perhaps most important, it became adapted to *Rumex paucifolius* Nutt. These two changes probably coincided, since *R. paucifolius* largely occurs in cool environments, presently in alpine environments. Both *L. rubidus* and *L. xanthoides* use large broad leaved *Rumex* species and not *R. paucifolius* (Ballmer & Pratt, 1989b). The geographic range of *L. editha* is entirely confined within the range of this food plant (Scott, 1986; Hitchcock & Cronquist, 1973; Scoggan, 1978). In areas where *L. editha* is not associated with *R. paucifolius*, as at Dunsmuir, the butterfly primarily uses the closely related species *Rumex acetosella* L. and/or *R. angiocarpus* Murb. These three *Rumex* species share similar floral and other characteristics, and key out in sequential couplets (Hitchcock & Cronquist, 1973). Both *R. acetosella* and *R. angiocarpus* are believed to be introduced from Europe (Munz, 1974). *Lycaena cupreus* (W. H. Edwards), a high altitude species also adapted to *R. paucifolius*, like *L. editha*, sometimes occurs at lower elevation in association with *R. acetosella* (Emmel & Pratt, pers. obs.).

A likely mechanism for these changes in a population of *L. x. dione*, involves entrapment during a glacial period in a blind prairie canyon or valley. This canyon must have been long, broad, and ran generally north-south. The blind end would have been blocked by mountains to the south, with high mountains to the east and the west blocking movement in all directions but the north. The closer to the southern edge of the glacier, the colder the canyon would have become. Such a prairie canyon occurs in the Rocky Mountains of Montana, the Red Rock River Canyon (Figure 7).

As the glacier moved south, probably *L. x. dione* moved south in response. Eventually the glacier may have even cut off any possible movement to the north, so that an isolated pocket of *L. x. dione* was formed. In such an isolated pocket the large *Rumex* species, on which *L. x. dione* was adapted, may have been gradually extirpated, being replaced by the colder adapted *R. paucifolius*. Therefore only those butterflies that went through a host shift to this new host survived.

The glacial period probably lasted a long time; for instance, the recent Wisconsin lasted for over 50,000 years (Martin & Mehringer, 1965). This is a lot of generations of selection upon a new host race. When the glacier receded, the new host race probably followed its food plant up into the Rocky Mountains, where it remained isolated from *L. x. dione* at high elevations. Probably during the following glacial period *L. editha* dropped down into the Great Basin desert (GB Figure 7), which was just south of

the Rocky Mountains of Montana. During the Wisconsin Glacial, subalpine plants extended down to basal elevations (Wells, 1983). At that time *L. editha* could have expanded its range throughout the Great Basin. Now *L. editha* is found throughout connecting mountain ranges that border the Great Basin desert.

L. x. xanthoides

Lycaena x. dione is adapted to the prairie habitat of the Great Plains and *L. x. xanthoides* is adapted to grassland habitats of California (SV in Figure 7). Although *L. x. xanthoides* does not occur exclusively in these grassland habitats, its range encompasses the region. Only one other Copper occurs in the grasslands of California, *Lycaena helloides* (Boisduval), and it also inhabits the prairie habitat of the Great Plains (Scott, 1986). It is possible, therefore, that *L. x. xanthoides* came from *L. x. dione* through a prairie corridor which led from the Great Plains to California. Because of the Rocky Mountains and Great Basin desert of the west, such a corridor probably would have had to have run from western Texas through southern New Mexico and Arizona (south of the White Mountains) and ended in the Mojave desert of California (WH and M in Figure 7).

Present distributions of three lycaenid species suggest that such a prairie corridor could have once existed, *Phaeostrymon alcestis* (W. H. Edwards), *Everes comyntas* (Godart), and *Lycaeides melissa* (W. H. Edwards) have populations adapted to the prairie habitats of the Great Plains. *Phaeostrymon alcestis* occurs through much of the southern regions of the Great Plains and its range overlaps that of *L. x. dione*. This butterfly occurs in 2 disjunct regions through this region from the White Mountains south (Scott, 1986). *Everes comyntas* also occurs throughout the Great Plains and in disjunct areas south of the White Mountains. This species extends its range into the grasslands of California and north into the grasslands of Washington and is absent from the Rocky Mountains and most of the Great Basin, and shows no likely east-west connection to the north (Scott, 1986). *Lycaeides melissa* which occurs through most of the west, occurs in this region south of the White Mountains as disjunct populations, whereas the butterfly's range outside of this region is largely continuous (Scott, 1986).

Although *L. x. dione* does not presently occur south to the gateway of this hypothetical prairie corridor, the northern half of its range was covered in glacier during the Wisconsin. It would seem likely that during the Wisconsin, *L. x. dione* probably extended south to this gateway. Not only would this prairie corridor have been cooler during a glacial period, but moister and more favorable to *Rumex* species. When the glacier retreated this corridor probably became too dry for *Rumex* species and the range of *L. x. dione* moved north. Now *L. x. dione* and *L. x. xanthoides* are isolated by the Rocky Mountains and Great Basin desert.

Conclusion

One of the remarkable results of this study are the number of statistical character differences found in different populations of the same species. Perhaps this is not too surprising in *L. rubidus* and *L. editha*, which historically may have formed isolated geographic populations during the Pleistocene, but these differences are even apparent in the first instars of different populations of *L. x. dione*. The Great Plains presently are rather uniform, with little if any geographic barriers to gene-flow. On the other hand, pockets of isolated prairie could have occurred during the Wisconsin glaciation. For instance, the eastern peninsula of grassland habitat of the Great Plains could have been isolated during a glaciated period (see Figure 7).

By increasing the number of carefully chosen populations, number of characters, and sample sizes, a more refined phylogenetic analysis could be performed which could answer more specific dispersal questions within the Editha Complex. Genitalic characters could also be examined and scored in much the same way as the larval characters, particularly since character differences have been observed in the *L. rubidus* subspecies (Johnson & Balogh, 1977). By increasing the sample sizes for some of the larval characters, greater statistical differences may be obtained which would create more characters that form significantly different classes. Such analyses may demonstrate vicariant events which could be timed by a known geological history of the regions. These analyses may also help illuminate potential vicariant events of other butterflies as well as other insects.

Acknowledgements. We thank Susan S. Borkin for ova of *L. x. dione* from Wisconsin, Jim Scott for ova of *L. editha* and *L. x. dione* from Colorado, Malcolm Douglas for ova of *L. r. ferrisi* from Arizona, Art Shapiro for females of *L. x. xanthoides* from Sacramento and ova of *L. heteronea* from Ball Mt (CA), Sterling Mattoon for the ova of *L. xanthoides* from Branscomb Lake (CA), Rudy Mattoni for ova of *L. xanthoides* from Acton and *L. heteronea* from Chuchupate Campground (CA), Adam Porter for ova of *L. xanthoides* from Silver Canyon (CA). Additional thanks are due to Adam Porter, Art Shapiro, and John Emmel for reading and commenting on an early version and two anonymous reviewers which helped in the final manuscript.

Literature Cited

- ANONYMOUS, 1986. Season Summary 1985, Colorado Section. News Lep. Soc. 2: 23.
- AUSTIN, G. T. & D. D. MURPHY, 1987. Zoogeography of Great Basin butterflies: Patterns of distribution and differentiation. Great Basin Naturalist, 47:186-201.
- BALLMER, G. R. & G. F. PRATT, 1989a. Instar number and larval development in *Lycaena phlaeas hypophlaeas* (Boisduval) (Lepidoptera:Lycaenidae). J. Lep. Soc. 43:59-65.
- , 1989b. A survey of the last instar larvae of the Lycaenidae (Lepidoptera) of California. J. Res. Lepid. 27: 1-80.

- . 1992. Quantification of Ant Attendance of Lycaenid Larvae. *J. Res. Lepid.* 30:95-112.
- CLARK, G. C. & C. G. C. DICKSON, 1956. Proposed classification of South African Lycaenidae from the early stages. *J. Ent. Soc. South Africa* 10, 195-215.
- CROWE, C. R., 1970. A possible new hybrid copper. *J. Res. Lep.* 8: 51-52.
- HAND, D. J. 1981. *Discrimination and Classification*, New York: John Wiley & Sons, Inc.
- HITCHCOCK, C. L. & A. CRONQUIST. 1973. *Flora of the Pacific Northwest*. University of Washington Press, Seattle Washington, pp. 90-92.
- JOHNSON, K. & G. BALOGH, 1977. Studies in the genus *Lycaena*. 2 Taxonomy and evolution of the Neararctic *Lycaena rubidus* complex, with description of a new species. *Bull. Allyn Museum* 43: 1-62.
- KLOTS, A. B., 1936. The interrelationships of the species of the genus *Lycaena* Fabricius (Lepidoptera, Lycaenidae). *Bull. Brooklyn Ent. Soc.* 31: 154-171.
- MARTIN, P. S., & P. J. MEHRINGER, JR. 1965. Pleistocene pollen analysis and biogeography of the Southwest. Pages 433-451 in H. E. Wright and D. G. Frey, editors. *The Quaternary of the United States*. Princeton University Press, Princeton, New Jersey, USA.
- MILLER, L. D., & F. M. BROWN, 1979. Studies in the Lycaeninae (Lycaenidae) 4. The higher classification of the American coppers. *Bull. Allyn Mus.* 51: 1-30.
- MUNZ, P. A. 1974. *A Flora of Southern California*, University of California Press, Berkeley, CA.
- PORTER, A. H. & A. M. SHAPIRO, 1991. Genetics and biogeography of the *Oeneis chryxus* Complex in California. *The Journal of Research on the Lepidoptera.* 28:263-276.
- PRATT, G. F. 1988. *The Evolution and Biology of Euphilotes Biotypes*. Unpublished doctoral dissertation, University of California, Riverside, 653 pp.
- SCOGGAN, H. J. 1978. *The Flora of Canada*, Bonnie Livingston, editor. National Museums of Canada, publisher, Ottawa, Canada, p. 639.
- SCOTT, J. A., 1980. Geographic variation in *Lycaena xanthoides*. *J. Res. Lep.* 18: 50-59.
- . 1986. *The Butterflies of North America*. Stanford University Press, Stanford, California.
- WELLS, P. V. 1983. Paleobiogeography of montane islands in the Great Basin since the last Glaciopluvial. *Ecological Monographs*, 53:341-383.
- WRIGHT, D. M., 1983. Life history and morphology of the immature stages of the bog copper butterfly *Lycaena epixanthe* (Bsd. & Le C.) (Lepidoptera: Lycaenidae). *J. Res. Lep.* 22: 47-100.